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INVITED ARTICLE

STRATEGIES TO IMPROVE PLASMA CIRCULATION OF NANOPARTICLES

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Abstract

There is a tremendous interest in developing long-circulating nanocarriers as treatment and imaging modalities. Enhanced plasma circulation of nanoparticles can improve on-target accumulation and help maintain sustained therapeutic drug levels in the plasma. Nanoparticles, however, are rapidly eliminated from systemic circulation by circulating and stationary macrophages. There have been several approaches devised to overcome this rapid elimination of nanoparticles. Reducing particle size has been shown to reduce opsonization and phagocytic uptake. Particle shape has also been shown to strongly affect the biological interactions of nanoparticles. Coating the surface of nanoparticles with hydrophilic polymers has been one of the most widely used strategies to protect against opsonization. Recent development of facile synthetic approaches has allowed researchers to engineer formulations that mimic blood cells. These strategies have also proven to be successful in producing long-circulating nanoparticles. This review provides a brief description of several approaches that have been used successfully to enhance the plasma circulation of nanoparticles.

Keywords: *RES, opsonization, long circulating, nanoparticles, PEG, biomimetic, particle size, particle shape*

Abbreviations: Mononuclear phagocytic system, MPS; reticuloendothelial system, RES; poly-(ethylene glycol), PEG; red blood cells, RBCs; monosialylganglioside, GM1; poly-(vinyl pyrolidone), PVP; poly-(acrylamide), PAA; poly-(acryloylmorpholine), PAcM; poly-(2-methyl-2-oxazoline), PMOZ; poly-(2-ethyl-2-oxazoline), PEOZ; poly-(lactide-co-glycolide), PLGA; accelerated blood clearance, ABC

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Introduction

Nano-sized carriers are rapidly emerging as a useful platform for drug delivery [1, 2]. While nanoparticles offer several advantages over other drug delivery systems, these advantages can be realized only if the carriers are maintained in circulation for prolonged time intervals.

For example, nanoparticles are extensively used to achieve targeted drug delivery [3, 4]. However, when the target has a limited blood supply or is at a very low abundance, nanoparticles need to circulate through the target several times in order to accumulate at high enough concentrations [5, 6]. Nanoparticles are also used as controlled-drug delivery vehicles to sustain high drug levels in the plasma. This too, can be achieved only if the nanoparticles remain in circulation for prolonged periods of time [7]. Hence, there is a considerable interest in improving the plasma residence time of nanoparticles. Once administered, nanoparticles are rapidly eliminated from blood circulation via a two-step process [8]. In the first step serum proteins, of the complement system, are adsorbed on the surface of nanoparticles. This process is known as opsonization and is vital for the elimination of nanoparticles [8-10]. Following opsonization, nanoparticles are engulfed by circulating macrophages or macrophages residing in the liver and spleen (referred to as mononuclear phagocytic system (MPS) or the reticuloendothelial system (RES)) [8, 9, 11]. Hence, in order to prolong the circulation time of nanoparticles, it is essential to subvert either of the two processes.

There have been several attempts to increase the blood circulation of nanoparticles [12, 13]. Initial attempts were directed towards altering the surface of particles [14]. This led to the use of non-ionic hydrophilic polymers [15]. This has been the most widely used strategy till date. Some researchers have also shown that changing the physicochemical properties of the core of the nanoparticles, like size and shape, can also increase their circulation half-life [16]. Recent advances in synthetic techniques have inspired researchers to develop nanoparticles that mimic blood cells [17]. These approaches have also helped prolong the plasma residence of nanoparticles.

In this short review, I have outlined a various strategies that have been used successfully to produce long circulating nanomedicine. I focus, primarily, on the rationale underlying the use of each technique, their advantages and potential shortcomings.

Modifying nanoparticle morphology to enhance circulation time

Particle size

Particle size is an important determinant of the plasma circulation time of nanoparticles [18]. Hence, altering the particle size can be an effective technique to prolong plasma circulation. However, unlike other strategies discussed in this review, the choice of particle size is

peculiar [19]. Nanoparticles are eliminated from plasma circulation via macrophage uptake, distribution to peripheral organs, or urinary excretion. Alterations in particle size can affect each of these processes [19]. Hence, careful consideration of particle size is required. The particle size at which all the three processes are minimized will result in the highest circulation time for nanoparticles. For spherical nanoparticles, macrophage uptake increases with an increase in hydrodynamic diameter. Fang *et al.* compared protein adsorption on 80, 170 and 240 nm nanoparticles incubated in mouse serum [20]. They found that 240 nm nanoparticles showed a 6-fold higher protein adsorption as compared to 80 nm particles. Consequently, larger nanoparticles had a higher macrophage uptake *in vitro*. Finally, 80 nm nanoparticles also showed a longer elimination half-life as compared to 170 and 240 nm nanoparticles [20]. Papović *et al.* compared the circulation half-life of 12, 60 and 125 nm nanoparticles [21]. They also found that the smallest particles had the longest elimination half-life. These results indicate that smaller particles are more efficient at evading the RES [21].

While smaller nanoparticles show lower RES uptake, decreasing the particle size below a certain threshold may result in elimination of particles through other mechanisms [19]. For example, Liu *et al.* showed that decreasing particle size below 50 nm increases their accumulation in the liver [22]. As hepatic capillaries are fenestrated (pore size ~75 nm), they are permissive to the entry of extremely small nanoparticles. Nanoparticles below 5.5 nm can be filtered through renal filtration [23]. Choi and colleagues showed that the circulation half-life of 8 nm quantum dots was longer than that of 4 nm quantum dots. This increased plasma residence for larger particles was because of the lower renal clearance of the larger nanoparticles [23]. Figure 1 shows the half-life of nanoparticles of various sizes [23, 24]. For extremely small particles (<20 nm), the half-life increases with an increase in particle size. The half-life reaches a peak at an intermediate particle size and then declines with a further increase in particle size. However, since RES uptake is often the major mechanism of particle elimination and most studies utilize particles in the size range of ~100 nm, smaller nanoparticles are generally considered favourable [24].

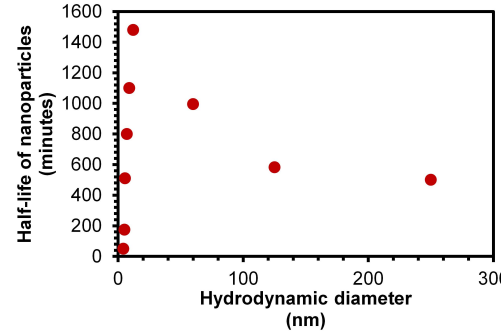

Figure 1: Half-life of various sized nanoparticles

The figure shows plasma half-life of nanoparticles of different sizes. For extremely small nanoparticles, renal clearance decreases with an increase in particle size. This leads to enhanced circulation times. For particles larger than 20 nm, RES uptake increases with an increase in particle size. Thus half-life decreases with an increase in particle size. Data was reported in [23, 24]. Adapted with permission from [23, 24].

Particle shape

There is very limited information available about the performance of non-spherical nanoparticles. This, in part, was due to lack of techniques for the synthesis of non-spherical nanoparticles. However, in recent years, synthesis and characterization of non-spherical nanoparticles has gained tremendous interest and has become a subject of extensive research [16, 25-27]. Geng and co-workers were the first to show that non-spherical nanoparticles have superior pharmacokinetics and anti-tumour efficacy as compared to spherical nanoparticles [28]. This thesis studied the plasma circulation of filamentous micelles composed of block co-polymers of poly-(ethylene glycol) (PEG) and poly-(ethylethylene) and that of poly-(ethylene glycol) and poly-(caprolactone). In a mouse model, filamentous micelles had a significantly longer half-life (~144 hours) than spherical polymerosomes (half-life ~24 hours). The effect of particle size of filomicelles was also studied. Interestingly, filomicelles having an initial length of 8 μm (equivalent to the diameter of red blood cells (RBCs)) had the longest circulation half-life. Filomicelles with a shorter or longer lengths were eliminated faster [28]. While the study by Geng *et al.* provided the first insight into the biological performance of non-spherical nanoparticles, it analysed only one particle shape. A mechanistic understanding of this interaction was first provided by Champion *et al.* [29]. This study focused on the macrophage uptake of polystyrene particles having a variety of shapes such as spherical, oblate ellipsoids, prolate ellipsoids, elliptical discs and rectangular discs. The authors found that the initiation and completion of phagocytosis were strongly affected by the morphology of the nanoparticles. The orientation of nanoparticles relative to the macrophages dictated the initiation of the process. The volume of nanoparticles (a function of particle size) dictated the completion of this process. For example, if the alignment of elliptical discs was perpendicular to the approaching surface of the macrophage, the process of phagocytosis did not initiate at all. However, if the elliptical disc was aligned parallel to the macrophage surface, phagocytosis initiated spontaneously. The completion of this process, however, depended on the volume of the particle relative to the macrophage [29]. Hence, particle shapes that minimize initiation of phagocytosis will have the longest circulation half-life.

Following these studies, there have been a number of experiments which have shown that certain non-spherical nanoparticles have superior pharmacokinetics, efficacy and targeting abilities as compared to spherical nanoparticles [30-33]. However, spherical nanoparticles are still preferred and more popular owing to their ease of synthesis.

Surface functionalization of nanoparticles with hydrophilic polymers

Opsonization and subsequent macrophage uptake of nanoparticles are known to be triggered by the hydrophobicity and charge of the surface of nanoparticles [9, 34]. Hence, initial attempts to decrease MPS mediated nanoparticle uptake were

directed towards masking the surface hydrophobicity and charge of nanoparticles. This has been achieved by using non-ionic hydrophilic polymers, physically adsorbed or chemically grafted, on the surface of nanoparticles.

The use of amphiphilic polymers was first suggested by Illum *et al.* [14]. In this study, polystyrene microparticles were surface functionalized with poloxamer 338, a tri-block co-polymer consisting of a central hydrophobic chain of poly-(propylene oxide), flanked by two hydrophilic chains of poly-(ethylene oxide). The authors showed that poloxamer decreased the rapid initial uptake of microparticles into the liver. This resulted in higher plasma concentrations at early time points. Although the hepatic uptake of particles was decreased considerably, there was no significant increase in terminal half-life of the surface coated particles [14].

In another study, Allen *et al.* hypothesized that increasing surface hydrophilicity and mimicking the cell surface of blood cells may enhance the circulation time of nanoparticles [35]. Earlier studies investigating the long circulation times of RBCs had highlighted the role of surface sialic acid moieties [36]. To replicate this, the liposome surface was modified with sialic acid by incorporating monosialylganglioside (GM1) into the liposome bilayer. The authors found that incorporation of GM1 in the lipid bilayer significantly decreased their hepatic uptake. As the molar ratio of GM1 in the lipid bilayer increased, RES uptake of liposomes decreased. However, increasing the amount of GM1 beyond a certain concentration compromised the stability of the lipid membrane [35]. This limitation could be overcome by using lipids, like sphingomyelin, which showed greater intermolecular interactions [37]. Such lipids increased the rigidity of the liposome membrane and allowed higher incorporation of GM1 [35]. Gabizon *et al.* later compared the tumour accumulation of non-functionalized liposomes and GM-1 functionalized liposomes in a mouse model of lymphoma [38]. GM-1 functionalized liposomes showed 25-fold increase in tumour levels as compared to the non-functionalized liposomes [38]. As GM-1 functionalized liposomes partially evaded the RES system, these liposomes were referred to as “stealth” liposomes.

Poly-(ethylene glycol)

Though GM-1 provided excellent stealth properties to nanocarriers, it was required in large quantities. Additionally, reproducible synthesis of this polymer was also challenging [39]. Hence, there was tremendous interest in developing alternate polymers to substitute GM1. Klibanov *et al.* used poly-(ethylene glycol) (PEG) to increase the circulation of liposomes [40]. This strategy had been successfully implemented to prolong the circulation half-life of proteins [41]. In agreement with those reports, the authors found that PEG functionalized liposomes (half-life: 5 hours) had a significantly higher plasma residence time than non-functionalized liposomes (half-life: 0.5 hours). GM-1 functionalized liposomes had an

intermediate half-life of 1.5 hours [40]. Papahadjopoulos *et al.* later confirmed these results in a mouse model of colon carcinoma [42]. PEG liposomes had an extended plasma half-life (~5 fold higher than the non-functionalized liposomes) and showed lower accumulation in the major RES organs, liver and spleen. Consequently, there was higher tumour accumulation of PEG-liposomes as compared to the plain liposomes [42].

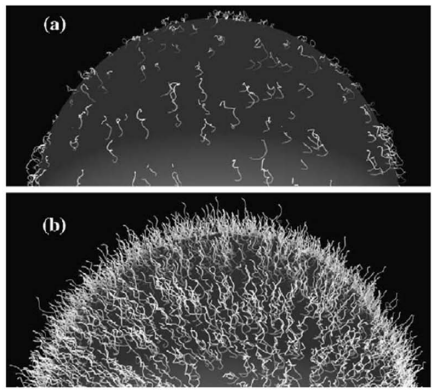

Figure 2: Schematic diagrams of PEG configurations on the upper hemisphere of a polymeric nanoparticle.

In (a), the low surface coverage of PEG chains leads to the "mushroom" configuration where most of the chains are located closer to the particles surface. In (b), the high surface coverage and lack of mobility of the PEG chains leads to the "brush" configuration where most of the chains are extended away from the surface. Reproduced with permission from [9]

PEG provides steric hindrance to the adsorption of opsonins and hence decreases the RES uptake of nanoparticles [9, 43, 44]. Surface density of PEG is an important variable that can affect the efficiency of the polymer [45]. Best steric hindrance is achieved at an intermediate density [46]. At a low surface density, PEG chains exist in a mushroom configuration, while at a high surface density they exist in a brush configuration (Figure 2). As opsonins approach the nanoparticle surface, they cause "brush"-shaped PEG chains to compress. The compressed-high energy conformation of PEG chains is not favourable and as it returns to the preferred elongated conformation, opsonins are pushed away from the nanoparticle surface. Hence, it is important that the surface density of PEG is high enough that they exist in a brush conformation. However, if the surface density is too high, PEG chains lose their flexibility and their protective ability. At low surface coverage, opsonins can get adsorbed in the space between the PEG chains [9, 45, 47].

Many studies have dealt with the effect of molecular weight on the efficacy of PEG [45, 48-50]. Higher molecular weight PEG provides better protection against opsonization [44]. Longer PEG chains can provide higher repulsive forces against the adsorption of opsonins [44]. However, functionalizing nanoparticles with extremely large PEG moieties decreases their interaction with the target cells as well [43]. Additionally, larger hydrophilic polymers are thermodynamically more stable when they are free in solution [51]. Thus, preparing stable nanoparticle formulations with larger PEGs is challenging. Hence, the use of mid-sized PEGs (molecular weight: 1000- 5000 Da) is most common [52, 53]. The method of PEGylation (surface adsorption vs. chemical grafting) and the type of PEG (branched vs. linear vs. star shaped) have

also been found to affect the efficiency of PEG. [54-58]. However, these parameters have not been discussed in this review.

PEG is the preferred polymer for making long circulating nanocarriers [6]. There are several reasons for the popularity of PEG. First, PEG is non-biodegradable. Hence, it does not produce any toxic metabolites and is easily cleared from blood circulation via renal filtration. Additionally, synthesis of PEG polymers with a low polydispersity index is relatively simple. This allows a precise control of the characteristics of the final product. Moreover, PEG chains are flexible. Hence a very small mole% of PEG is required to cover the entire nanoparticle surface and provide stealth properties to the formulation [6, 47]. Finally, the two terminal groups of PEG can be tailored to specification. It is common to conjugate one end group with a hydrophobic polymer (to allow docking on the lipophilic surface of the nanoparticle) and the other end group to targeting moieties [3, 52, 53].

Other polymers

Due to the success of PEG, there was considerable interest in investigating other hydrophilic polymers for stealth properties. Vinyl polymers like poly-(vinyl pyrolidone) (PVP), poly-(acrylamide) (PAA) and poly-(acryloylmorpholine) (PAcM) were one of the first alternate polymers studied [51, 59]. PVP, PAA and PAcM were conjugated to a hydrophobic phospholipid or acyl chain to produce amphiphillic derivatives. Molecular weights of the acyl linker and PVP or PAA were optimized to provide highest encapsulation efficiency and steric protection. However, it was found that these polymers performed similar to PEG and did not provide any additional benefit [51, 59].

Amphiphillic derivatives of poly-(2-methyl-2-oxazoline) (PMOZ) and poly-(2-ethyl-2-oxazoline) (PEOZ) have also been investigated [60]. It was found that both, PEOZ and PMOZ, delayed RES clearance of liposomes in a rat model. The half-life of PEOZ and PMOZ functionalized liposomes was similar to that of PEG functionalized liposomes [60]. Maruyama and colleagues showed that polyglycerols could also be used to delay RES uptake of liposomes [61]. Amphiphillic derivatives of polyglycerol were synthesized by conjugating polyglycerol with dipalmitoylphosphotidic acid. The presence of polyglycerol moieties on the surface of liposomes decreased their uptake in major RES organs like the liver, kidney and spleen. The protective ability of polyglycerol increased with the degree of polymerization of the polymer. However, this study did not compare the polyglycerol derivatives to PEG [61].

Some studies have also shown that polysaccharides can be used effectively to provide stealth properties to nanoparticles [62]. In particular, dextran coating has been widely used to protect iron oxide nanoparticles against RES uptake [63]. Polysaccharides provide a unique advantage over other polymer coatings. Polysaccharides can be used as ‘active targeting’ ligands, as many cells express

receptors for these molecules [62]. While PEG and other synthetic polymers are widely accepted [64-66], there are certain disadvantages associated with them. Some reports have shown the production of anti-PEG IgM antibodies after the first dose of PEGylated nanoparticles [67, 68]. This significantly enhances the blood clearance of the second dose of nanoparticles. In fact, the second dose of PEG nanoparticles are eliminated almost as rapidly as non-functionalized nanoparticles. This phenomenon, termed as accelerated blood clearance (ABC) [67], can have significant ramifications on the use of synthetic polymers. It should be noted, however, that ABC is observed only for a limited time after the first dose of PEGylated nanoparticles. Ishida *et al.* found that if the second dose of PEG nanoparticles is administered 3-7 days after the first, there is 3-5-fold increase in the liver accumulation of second-dose-nanoparticles as compared to the first dose. However, if the second dose is administered two weeks after the first dose, there is no difference in the liver accumulation of the nanoparticles [69]. Thus allowing sufficient time between consecutive doses of PEGylated nanoparticles can help overcome the problem of ABC.

Biomimetic approaches to enhance plasma circulation of nanoparticles

RES mediated uptake of nanoparticles can be significantly reduced by using hydrophilic polymers. But a major fraction of the dose is still found in the liver and spleen. Additionally, immune reaction upon repeated dosing limits the utility of this approach. Hence, alternate approaches for engineering stealth nanoparticles are of tremendous interest. Most nanoparticulate systems show a circulation half-life of ~10-12 hours. In contrast, blood cells (like erythrocytes) remain in circulation for weeks. Thus, several studies are aimed at generating nano-drug delivery systems that mimic blood cells.

Modifying the surface of nanoparticles to mimic blood cells

The mechanism of RES evasion by RBCs is a subject of extensive research. Though RBCs undergo opsonization they are not engulfed by macrophages. RBCs employ various self-markers to evade RES uptake. These include complement receptor 1, decay accelerating factor, C8 binding protein, CD59 and CD47 [70]. CD47 is expressed on the plasma membrane of erythrocytes [71] and often upregulated on circulating tumour cells and stem cells [72, 73]. CD47 interacts with signal receptor protein α (SIRPα), an inhibitory receptor found on the surface of macrophages. Activation of SIRPα leads to an intracellular cascade resulting in the inactivation of myosin. This retards macrophage mediated phagocytosis [73]. Hence, activating SIRPα on the macrophage surface can reduce the phagocytosis of nanoparticles.

Tsai *et al.* were the first to explore this approach [74]. In this report, streptavidin coated polystyrene microparticles were conjugated to biotinylated CD47. *In vitro* studies showed that, in spite of similar levels of opsonization, CD47 coated

microparticles had a significantly lower macrophage uptake than non-functionalized microparticles. Additionally, macrophage uptake decreased with an increase in the surface density of CD47, reaching a plateau at ~200 molecules/μm^2 [74]. Rodriguez *et al.* tested whether presence of CD47 on the surface of 160-nm polystyrene nanoparticles increases their circulation time *in vivo* [75]. The authors found that CD47 functionalized nanoparticles had a longer plasma circulation as compared to the non-functionalized nanoparticles. It is interesting to note that CD47, unlike PEG, has no effect on the opsonization process. CD47 is involved in decreasing phagocytosis via macrophages. Thus, in principle, presence of PEG and CD47 can offer a dual advantage. Consistent with this hypothesis, the authors found that PEGylated polystyrene nanoparticles functionalized with CD47 had a longer plasma residence time and showed lower accumulation in the spleen as compared to PEGylated polystyrene nanoparticles [75].

The presence of CD47 on the surface of nanoparticles improves their plasma circulation. However, the circulation time of these nanoparticles is still not comparable to blood cells. It is possible that the activity of CD47, alone, cannot provide nanoparticles with stealth properties like blood cells. Surface density of CD47 and the nature of interaction of CD47 with SIRPα may also be different for these artificial vessels. In other words, it is difficult to recreate the complex biochemistry of blood cells on the surface of nanoparticles using a single ligand. To overcome this issue, some recent studies have proposed coating nanoparticles with the entire plasma membrane of blood cells.

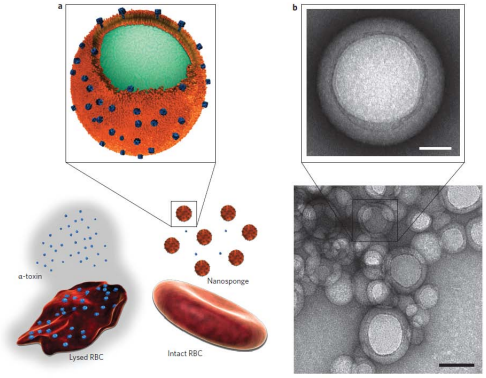

Figure 3: Schematic and actual structures.

a, Schematic structure of toxin nanosponges and their mechanism of neutralizing toxins. The nanosponges consist of substrate-supported RBC bilayer membranes into which toxins can incorporate. After being absorbed and arrested by the nanosponges, the toxins are diverted away from their cellular targets, thereby avoiding target cells and preventing toxin-mediated haemolysis. b, TEM visualization of nanosponges mixed with a-toxin (scale bar, 80 nm) and the zoomed-in view of a single toxin-absorbed nanosponge (scale bar, 20 nm). The sample was negatively stained with uranyl acetate before TEM imaging.
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Hu *et al.* coated poly-(lactide-*co*-glycolide) (PLGA) nanoparticles with the plasma membrane of RBCs [76]. The authors confirmed that major proteins in the RBC membrane were retained even after the coating process. In mouse studies, erythrocyte membrane coated nanoparticles showed an elimination half-life of ~40 hours while PEG nanoparticles showed a half-life of ~15 hours. RBC-coated nanoparticles were later used as decoys to adsorb and eliminate blood

borne-toxins that can cause haemolysis (Figure 3) [77]. In this study, the surface of nanoparticles attracted the blood-borne toxin, while the core of the nanoparticle acted as a 'sponge' to absorb the toxic chemical. This membrane-coating platform has been applied successfully to other nanoparticle systems as well [70, 78].

Parodi *et al.* coated nanoporous silicon microparticles with leukocyte cell membrane [79]. The camouflaged microparticles, termed as leuko-like vectors, showed significantly reduced opsonization and macrophage uptake *in vitro* as compared to plain microparticles. Membrane coating also reduced the *in vivo* hepatic uptake of the microparticles. However, this advantage was transient. While the initial hepatic uptake of coated microparticles was lower, both coated and uncoated microparticles showed similar levels of hepatic uptake 40 minutes post injection. This indicates that the particles lose their coating rapidly and accumulate in the liver. In spite of this, coated microparticles showed a higher tumour accumulation as compared to the uncoated particles [79].

Blood cell mimicking nanoparticles show a longer half-life than PEGylated nanoparticles. However, most PEGylated nanoparticles are produced using single-step processes [53, 66]. Thus PEGylation is a simpler and more reproducible technique than the techniques mentioned above. Additionally, availability of erythrocytes and leucocytes may also significantly limit the translation of these approaches.

Altering the core of nanoparticles to mimic the flexibility of blood cells

Prolonged circulation of RBCs is also attributed to the structure and flexibility of these cells. Erythrocytes are too large to pass through constricted capillaries of the lungs. However, RBCs can pass through capillaries that have a diameter as small as 5μm [80]. Similar to RBCs, circulating tumour cells are also known to be more flexible as compared to normal cells. This flexibility allows circulating tumour cells to extravasate into, intravasate out of circulation and avoid getting trapped in constricted capillaries [81]. Thus, engineering the flexibility of long circulating cells into nanoparticles may allow longer plasma circulation.

Doshi and colleagues showed that the shape and flexibility of RBCs can be built into PLGA microparticles [82]. Spherical PLGA particles were converted to discoid biconcave structures using partial fluidization. Using layer-by-layer coating, bovine serum albumin and haemoglobin were added to the surface of the discoid microparticles. The particles were again exposed to a solvent to fluidize the core even further. The resultant particles were extremely soft and had a remarkably reduced elastic modulus. The pharmacokinetics of these particles was not studied in this report [82]. Merkel *et al.* showed that the plasma residence of 2-hydroxyethyl acrylate microparticles can be changed by altering their bulk modulus [83]. Microparticles were synthesized by cross linking 2-hydroxyethyl acrylate with poly-(ethylene glycol) diacrylate. Bulk

modulus of the microparticles was controlled by modifying the amount of cross-linker in the formulation. The authors found that by changing the modulus from 63 kPa to 8 kPa, the elimination half-life of the microparticles increased from 3 hours to 93 hours. This dramatic increase in half-life was attributed to the deformability of the microparticles and their ability to escape constricted microvasculature. It is interesting to note that though this study utilized microparticles, the half-life achieved was greater than some nanoparticulate systems [83]. Haghgooie *et al.* also showed that similar flexible microparticles can be produced in a variety of shapes and carrying different cargoes [84].

Conclusion

The plasma circulation time of nanoparticle is a key feature that dictates the final performance of these carriers. Rapid macrophage uptake of nanoparticles severely reduces their circulation time. There have been several attempts to reduce RES uptake of nanoparticles. These strategies include modifying particle size, shape, surface characteristics etc. More recently, several studies have attempted to produce nanoparticles that better mimic naturally occurring colloids like RBCs. In spite of considerable improvement in the plasma circulation of nanoparticles, most systems still show a high accumulation in the RES organs like the liver and spleen. Hence, alternate strategies are still of considerable interest in the field of medical nanotechnology.

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